

CLAIMS

1. A method of screening for protein secreting recombinant host cells comprising screening for promoter activity of a stress inducible promoter.
2. The method according to claim 1 comprising the steps of
 - (i) Providing a host cell comprising the stress inducible promoter operably linked to nucleic acid sequence encoding a reporter protein or a regulator protein.
 - (ii) Providing a nucleic acid sequence of interest.
 - (iii) Introducing the nucleic acid sequence in (ii) into the host cell in (i)
 - (iv) Culturing host cell obtained in (iii) under conditions promoting secretion of the protein encoded by the nucleic acid sequence from (ii); and
 - (v) Selecting the host cell exhibiting the desired level of reporter protein expression.
3. The method according to claim 2, wherein the regulator protein controls the expression of the reporter gene.
4. The method according to claim 3, wherein the regulator protein is an activator or repressor of the expression of the reporter protein.
5. The method according to claim 1, where the host cell is selected from bacterial cells.
6. The method according to claim 5, where the host cells belong to a strain selected from the group consisting of the species *Bacillus alkalophilus*, *Bacillus agaradhaerens*, *Bacillus amyloliquefaciens*, *Bacillus brevis*, *Bacillus clausii*, *Bacillus circulans*, *Bacillus coagulans*, *Bacillus lautus*, *Bacillus lentus*, *Bacillus licheniformis*, *Bacillus megaterium*, *Bacillus stearothermophilus*, *Bacillus subtilis*, *Bacillus thuringiensis*, *Streptomyces lividans*, *Streptomyces murinus*, *Escherichia coli*, *Lactococcus lactis*, and *Pseudomonas putida*.
7. The method according to claim 1, where the stress inducible promoter is comprised by the nucleic acids 1-999 of SEQ ID NO.:1.
8. The method according to claim 1, where the stress inducible promoter comprises the nucleic acids 1-999 of SEQ ID NO.:1.

9. The method according to claim 1, where the stress inducible promoter consists of the nucleic acids 1-999 of SEQ ID NO.:1.
10. The method according to claim 1, where the stress inducible promoter in its normal position regulates a gene encoded protein that is a functional homolog of the gene encoded protein regulated by the promoter sequence comprised by nucleic acids 1-999 of SEQ ID NO.:1.
11. The method according to claim 1, where the stress inducible promoter in its normal position is the promoter linked to a gene encoding a polypeptide which has at least 70% identity to the amino acid sequence of SEQ ID NO.:2.
12. The method according to claim 1, where the stress inducible promoter is the promoter linked to a gene encoding a polypeptide which has at least 80% identity to the amino acid sequence of SEQ ID NO.:2, or at least 90% identity to the amino acid sequence of SEQ ID NO.:2, or at least 95% identity to the amino acid sequence of SEQ ID NO.:2, or at least 98% identity to the amino acid sequence of SEQ ID NO.:2.
13. The method according to claim 1, where the stress inducible promoter is comprised by the repeated octameric motif of SEQ ID NO.: 3.
14. The method according to claim 1, where the stress inducible promoter comprises the repeated octameric motif of SEQ ID NO.: 3.
15. The method according to claim 1, where the stress inducible promoter is identical to the octameric motif of SEQ ID NO.: 3.
16. The method according to claim 2, where the reporter protein is 2-fold over expressed in a secretion stressed cell compared to a non secretion stressed cell, preferably 5-fold over expressed in a secretion stressed cell compared to a non secretion stressed cell, more preferably 10-fold over expressed in a secretion stressed cell compared to a non secretion stressed cell, or 20-fold over expressed in a secretion stressed cell compared to a non secretion stressed cell, most preferably 50-fold over expressed in a secretion stressed cell compared to a non secretion stressed cell, or more than 100-fold over expressed in a secretion stressed cell compared to a non secretion stressed cell.

17. The method according to claim 2, where the reporter protein is selected from the group consisting of fluorescent protein, antibiotic markers, and substrate converting enzymes.
18. The method according to claim 1, where the stress inducible promoter is comprised by
5 nucleic acids 1-999 of SEQ ID NO.:1, and the host cell further comprises an IPTG-inducible promoter operably linked to a nucleic acid sequence encoding the amino acids 1 to 449 of SEQ ID NO:2.
19. The method according to claim 1, where the stress inducible promoter comprises
10 nucleic acids 1-999 of SEQ ID NO.:1, and the host cell further comprises a IPTG-inducible promoter operably linked to a nucleic acid sequence encoding the amino acids 1 to 449 of SEQ ID NO:2.
20. The method according to claim 1, where the stress inducible promoter consists of
15 nucleic acids 1-999 of SEQ ID NO.:1, and the host cell further comprises a IPTG-inducible promoter operably linked to a nucleic acid sequence encoding the the amino acids 1 to 449 of SEQ ID NO:2.
21. The method according to claim 2, where the nucleic acid sequence in 2(ii) encodes a
20 polypeptide.
22. The method according to claim 2, where the nucleic acid sequence in 2(ii) encodes an enzyme.
23. The method according to claim 22, where the enzyme is selected from the group
25 consisting of proteases, cellulases (endoglucanases), beta-glucanases, hemicellulases, lipases, peroxidases, laccases, alfa-amylases, glucoamylases, cutinases, pectinases, reductases, oxidases, phenoloxidases, ligninases, pullulanases, pectate lyases, xyloglucanases, xylanases, pectin acetyl esterases, polygalacturonases,
30 rhamnogalacturonases, pectin lyases, mannanases, pectin methylesterases, cello-biohydrolases, transglutaminases and phytases.
24. The method according to claim 21, where the nucleic acid sequence is obtained by
35 mutating a nucleic acid sequence encoding a polypeptide.
25. The method according to claim 21, where the nucleic acid sequence is obtained by mutating a nucleic acid sequence encoding a protein engineered polypeptide.